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FEEDING OF SOIL MICROBIAL COMMUNITY ON ORGANIC MATTER FROM ITS DEAD CELLS*

ABSTRACT: Model experiments were conducted in incubated soil in order to compare the assimilation by microorganisms of the organic substance of their dead cells and the assimilation of plant residues. In culture experiments the effect of carbon and nitrogen concentration in the breeding-ground on the rate and efficiency of cell resynthesis was being observed as well as the formation of microorganism community in the presence of a large number of dead cells.

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1. INTRODUCTION

In previous investigations (Kaszubiak, Kukielska and Kaczmarek in press) it has been observed that there is constant multiplication and death of cells of microorganism community in soil. Thus during the growth season of plants this community is several times renewed.

Using methods of Arištovskaja (1972) it has been calculated that the produced biomass of microorganisms is dozen tons or so of dry mass/ha per year. Plant and animal remains and manure do not cover the requirement of microorganisms for organic matter for synthesis. Thus, one has to assume that there are additional sources of food for the microorganisms in soil. These are metabolites and dead cells.

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Here, the ability of microorganisms community to decompose its own dead cells and to resynthetize depending on the carbon and nitrogen content in the breeding ground, has been investigated. The ability to assimilate organic substance by microorganisms from their dead cells and plant residues was compared. The contribution of particular physiological and taxonomic groups in this process was observed. The study was conducted in 1974.

2. MATERIAL AND METHODS

2.1. Culture experiments

A liquid medium made on soil exctract after Lochhead (1940) with 0.1% yeast extract added was inoculated with soil suspension introducing about 2×10^3 microorganisms per 1 ml of medium. The culture was in a rotatory thermostat for 5 days at 26°C, the cells were centrifuged and after rinsing them 3 times they were suspended in a solution of mineral salts acc. to Hattori (1973). This solution contained in 1 litre : 100 mg KCl, 200 mg $MgSO_4 \cdot 7H_2O$, 50 mg $CaCl_2 \cdot 2H_2O$, 25 mg $FeSO_4 \cdot 7H_2O$, 5 mg Na_2MoO_4, 0.5 mg $MnSO_4 \cdot 4H_2O$, 0.5 mg $NaWO_4 \cdot 2H_2O$, 0.5 mg $ZnSO_4 \cdot 7H_2O$. A suspension containing 1.6×10^9 cells/ml was made and 50 ml of it was poured into 300 ml Erlenmayer flasks and the cells were killed at 120°C for 20 minutes. After sterilization, into some flasks the sterile solution $(NH_4)_2SO_4$ was added to the final concentration 0.15 mg $N{-}NH_4$/ml, whereas to some flasks the glucose was added to the final concentration 0.75 mg C/ml. Nothing was added to the remaining flasks. The contents of all flasks were inoculated with soil suspension by introducing 2.2×10^{-3} microorganisms/ml of the flask content. Incubations were conducted in the rotatory thermostat at 26°C for 132 h.

Every 12 h the numbers and biomass of multiplied cells were determined in the cultures to grasp the moment when both parameters will attain their maximum.

The numbers were determined by the plate method using the soil-extract medium.

The biomass was indirectly determined on the basis of the dimensions of cells of microorganisms growing in pure cultures obtained from colonies grown on plates in estimations of numbers. Therefore, in each experimental variant 100 colonies of microorganisms were picked at random and their cells were introduced into the semi-solid medium made of soil extract with yeast extract added. After 5 days of incubation at 26°C the size of microorganisms was measured in stained microscope preparations. Knowing the dimension of cells their weight was calculated assuming the specific weight of microorganisms as 1.1 g/cm^3. In calculations of the dry mass of cells it was assumed that they contain 80% of water.

In isolated pure cultures of microorganisms some physiological properties were determined and the presence of *Arthrobacter* was checked. Therefore, cultures isolated from the plates for counting of microorganisms inoculation, made after 4 days of inoculum multiplication on dead cells, were chosen because the numbers and biomass of multiplie microorganisms was then the highest. The following were examined: proteolytic properties – on soil medium with gelatine, amino-acids requirement using West and Lochhead method (1940), ammonification power – on soil medium with an addition of 0.2% asparagine, using Nessler's reagent ; nitrates assimilation – on medium with nitrates made acc. to West and Lochhead (1940).

2.2. Model experiments with incubated soil

Sieved sandy-loamy soil from an experimental field at Rogaczewo near Kościan was incubated in 3-litre jars, in 2 kg samples. The experimental variants were as follows: soil without any additions (control), soil with an addition of 2 g dry mass of *Escherichia coli* killed by heat (105°C) per 1 kg soil, soil with an addition of 2 g dry mass ground sugar-beet leaves per 1 kg soil.

Incubation was carried at 26°C, at constant soil moisture, determining the total number of microorganisms, proteolytic bacteria and bacteria lysing dead cells of *Escherichia coli* after 0, 1, 3, 7, 14 and 21 days.

For direct determinations the fluorescent microscope and acridine orange acc. to Strugger (1948) were used.

The numbers of proteolytic bacteria were determined on a medium with soil extract and gelatine. For determination of the numbers of bacteria lysing dead cells of *E. coli* the water agar with an addition of such a number of cells which produce distinct turbidity of the medium was used. The colonies around which the zones of brightening up of the medium occurred were counted.

3. RESULTS AND DISCUSSION

The results of culture experiments given in Table I show that when the dead cells were the only source of carbon and nitrogen in the medium, about 1/3 of their mass became resynthetized. The addition of N-NH_4 increased only slightly the number of produced cells, but distinctly increased their mass as the cells multiplied under these conditions were bigger. The efficiency of resynthesis of dry mass reached 57%.

Table I. Resynthesis of cells of soil microorganisms in cultures

Medium	Dead cells in the medium		Number of multiplied cells		Resynthesis efficiency (in %) calculated on the basis of:	
	numbers (10^9/ml)	dry mass (mg/ml)	numbers (10^9/ml)	dry mass (mg/ml)	numbers of cells	dry mass of cells
Suspension of dead microorganisms(SDM)	1.60	0.20	0.45	0.07	28.4	34.1
SDM + ammonium sulphate	1.60	0.20	0.53	0.12	33.1	56.6
SDM + glucose	1.60	0.20	1.92	0.33	119.4	162.4

Introduction of glucose to the medium as an additional source of organic carbon resulted in a greater number of multiplied cells than the number of introduced dead cells. This shows that organic carbon is the main factor limiting the utilization of chemical compounds in dead cells for the renewal process of microorganisms communities.

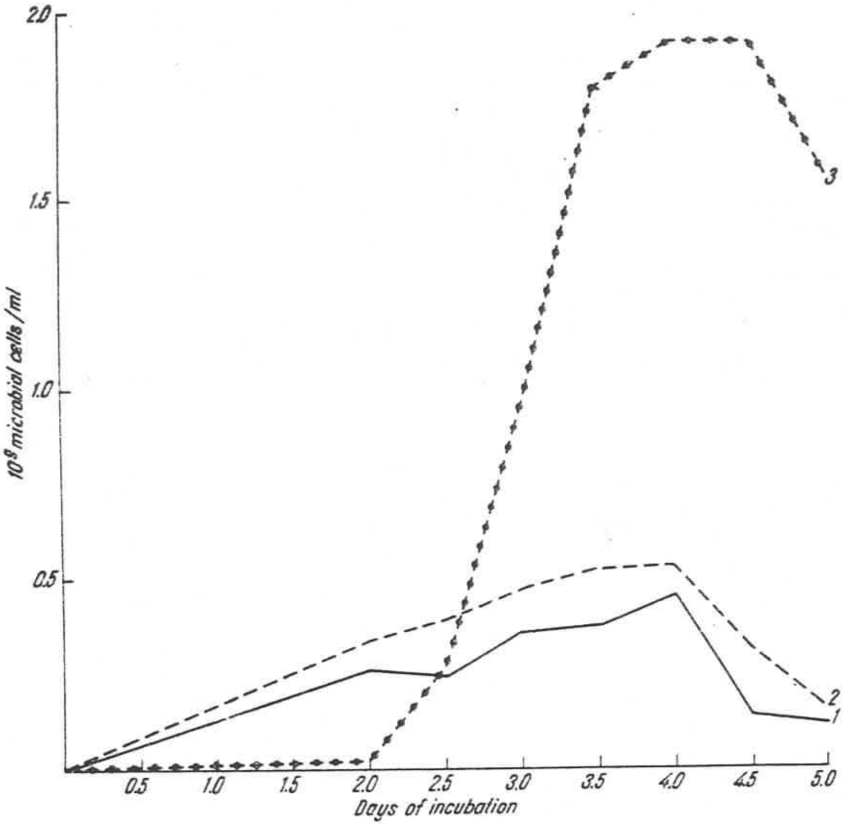

Fig. 1. Dynamics of resynthesis of cells of soil microorganisms in cultures
1 – suspension of dead cells (SDC), *2* – SDC + ammonium sulphate, *3* – SDC + glucose

In these experiments the maximum values for resynthesis were found in 4-day cultures (Fig. 1). Chemical compounds of dead cells are relatively quickly released and transformed into live plasma.

The susceptibility of microorganisms to decomposition can be illustrated by the results of studies on the susceptibility to oxidation of bacterial cells and plant remains (see Table II). Cells of *Escherichia coli* are more intensly oxidated than sugar-beet leaves which are considered as plant material easily assimilated by microorganisms (Muszyńska and Andrzejewski 1974).

Table II. Susceptibility to oxidation of *Escherichia coli* cells and plant residues

Material	mg of used O_2/g dry mass*
E. coli cells	42.6
Sugar-beet leaves	39.3
Rye straw	28.4

*100 mg dry mass of material was incubated in 50 ml 0.005 n $KMnO_4$ for half an hour.

Results of model experiments (Table III) show that in the presence of dead bacteria cells the microorganisms multiply to a greater extent than when adding the plant material. This mainly concerns the microorganisms growing on plates, which according to Kaczmarek, Kaszubiak and Guzek (1973) belong to the zymogenic microflora.

Table III. The effect of added dead *E. coli* cells and ground sugar-beet leaves on the occurrence of microorganisms in soil

Soil	Total numbers based on:		Numbers of proteolytic bacteria (10^6/g dry mass)	Numbers of bacteria lysing dead *E. coli* cells (10^6/g dry mass)
	plate method (10^6/g dry mass)	direct method (10^9/g dry mass)		
No additions (control)	11	3.0	0.06	0.18
+ *E. coli*	54	5.0	0.40	0.87
+ Sugar-beet leaves	34	4.8	0.14	0.77

*Mean values of analyses made at 5 dates during 21 days of soil incubation are given.

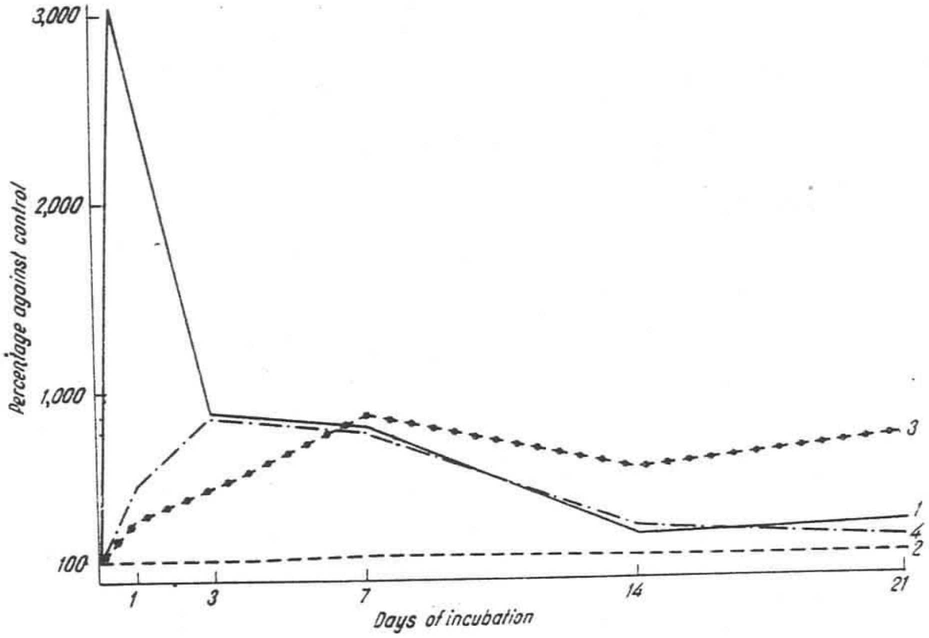

Fig. 2. Development dynamics of various groups of microorganisms in soil incubated with an addition of sugar-beet leaves

1 – total number of microorganisms determined by plate method, *2* – total number of microorganisms determined by direct method, *3* – numbers of proteolytic bacteria, *4* – numbers of bacteria lysing dead *E. coli* cells

It is worth noticing that the development of bacteria lysing dead cells of *E. coli* is stimulated not only by the addition of these cells to soil, but also by the addition of plant material. The quick reaction of these bacteria when adding organic substance (Fig. 2) shows that in microbiological studies they can be an indicatory group.

At a high number of dead cells in the medium there can be other changes than described in the community of microorganisms. In cultures with an addition of dead cells the microorganisms actively participating in nitrogen transformations, namely proteolytic bacteria – requiring amino-acids for growth, ammonifiers and bacteria assimilating nitrates, tend to increase their contribution to the community (Table IV). Ninety per cent of strains of bacteria assimilating nitrates show proteolytic properties, which explains their intense development on dead cells.

Table IV. Occurrence of active bacteria in nitrogen transformations in a culture multiplied on dead cells

Medium	Proteolytic bacteria (in 10^6/ml)	Bacteria requiring amino-acids for growth (in 10^6/ml)	Ammonifiers (in 10^6/ml)	Bacteria assimilating nitrates (in 10^6/ml)
Suspension of dead microorganisms (SDM)	346 (76)*	150 (33)	455 (100)	214 (47)
SDM + ammonium sulphate	403 (76)	244 (46)	520 (98)	207 (39)
SDM + glucose	115 (6)	57 (3)	1,858 (97)	690 (36)
Inoculum: diluted soil suspension	0.00070 (32)	0.00013 (6)	0.00134 (61)	0.00057 (26)

*Figures in brackets are given in %.

Taxonomic studies on microorganisms growing on dead cells have been also carried out, paying the greatest attention to the occurrence of *Arthrobacter*. According to Hattori (1973) particular bacteria species vary in their ability to resynthetize, which is observed to a great extent among the *Arthrobacter* genus. In our experiments no tendency was observed to selection of specific taxonomic groups. The percentage of *Arthrobacter* cells in cultures with an addition of dead cells was more or less the same as in the inoculum.

4. SUMMARY

Experiments with cultures and incubated soil were carried out in order to determine the ability of community of soil microorganisms to decompose its dead cells and to use this substance for a resynthesis of live cells.

Soil microorganisms used their own dead cells much better than the plant residues (Tables II, III). The process of resynthesis of microorganism cells was a quick one (Fig. 1) and its efficiency depended on the presence of additional sources of carbon and nitrogen in the medium (Table I). Organic carbon was the main

factor limiting the utilization of dead cells for the renewal of microorganism community. Its sufficient amount in the medium enabled a full resynthesis. The presence of numerous dead cells resulted in higher contribution of groups active in nitrogen transformations in the community of soil microorganisms (Table IV).

5. POLISH SUMMARY (STRESZCZENIE)

W przedstawionej pracy prowadzono doświadczenia na pożywkach i z glebą inkubowaną. Doświadczenia miały na celu określenie zdolności zespołu drobnoustrojów glebowych do rozkładu obumierających w nim komórek i wykorzystania powstałej substancji do resyntezy komórek żywych.

Stwierdzono, że drobnoustroje glebowe lepiej wykorzystywały jako pokarm swoje martwe komórki niż resztki roślinne (tab. II, III). W przeprowadzonych doświadczeniach resynteza komórek drobnoustrojów przebiegała szybko (fig. 1), a jej wydajność zależała od obecności w podłożu dodatkowych źródeł węgla i azotu (tab. I). Węgiel organiczny był głównym czynnikiem ograniczającym wykorzystanie martwych komórek do odnawiania się zespołu drobnoustrojów. Przy odpowiedniej zawartości w podłożu zapewniał on pełną resyntezę. W obecności licznych martwych komórek powstawały zmiany w zespole drobnoustrojów glebowych w kierunku zwiększania w nim udziału grup aktywnych w przemianach azotu (tab. IV).

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